Brief Definitive Report

THE ROLE OF POLYMORPHIC I-A^k β CHAIN RESIDUES IN PRESENTATION OF A PEPTIDE FROM MYELIN BASIC PROTEIN

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Immune response (Ir) genes of the MHC are highly polymorphic and generally control both the cellular and humoral responses to antigenic challenge (1). For class I gene-encoded molecules, most of the polymorphic residues are located around the proposed antigen binding site (2). A hypothetical model of class II molecules predicts that this site would be made up of the combined membrane-distal domains of both α and β chains (3).

How does the extensive polymorphism displayed by MHC molecules affect their function? In the absence of structural data for class II molecules, various groups have proceeded to test either spontaneous (4) or selected (5–8) mutants of class II encoded molecules for function in antigen presentation assays. In summary, the findings imply that amino acid substitutions at positions scattered throughout the membrane distal domains of both α and β chains can affect the presentation of antigen to T cells. However, in these studies, it was not always possible to distinguish antigen binding from T cell interaction defects. Here we report an analysis of the presentation of a single peptide epitope of myelin basic protein to a panel of MBP-specific, I-A^k-restricted T helper cell clones using cells expressing I-A^k molecules with mutations at defined residues of the β chain. The results show that the polymorphic regions of the I-A^k β chain can accommodate a number of amino acid changes without abrogating binding of this peptide. However, isolated substitution of amino acids in these regions can drastically alter the pattern of T cell clones capable of interacting with the MHC/antigen complex.

Materials and Methods

T Cell Clones. The isolation of T cell clones specific for the NH_2-terminal nine amino acids of rat MBP (rMBP) will be described in detail elsewhere (Davis, C. B., et al. manuscript submitted for publication). Briefly, T cells were isolated from two I-A^k mouse strains of distinct genetic backgrounds, B10.A(4R) and A/J, after immunization with a peptide corresponding to the NH_2-terminal eleven amino acids of rMBP. After three restimulations in vitro, clones were isolated from one A/J cell line and one B10.A(4R) cell line by sorting on

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the FACS II flow cytometer, using propidium iodide and forward angle light scatter to select only single viable cells. In contrast to clones AJ1.2 and 4R3.7, which are I-A^k restricted, clones 4R3.9, 4R3.4, and 4R3.6 respond to rMBP 1-16 presented by both I-A^k and I-A^u; clones 4R3.4 and 4R3.6 are also alloreactive on I-A^u. None of the clones show antigen-dependent or alloreactive responses to either I-A^d or I-A^s. We have not noted any distinction in either MHC recognition or peptide fine specificity to correlate with the genetic background from which the T cells were derived.

Antigen-presenting Cells. The cell lines used as APC for this study have been described in detail elsewhere (9). Briefly, either wild-type α and β chains or site-directed mutants of the β chain of I-A^k were transferred into an I-A β chain-negative variant of M12, M12.C3 (10), by electroporation. Transfected cell lines were appropriately selected and analyzed for cell surface expression of wild-type or mutated I-A by quantitative immunofluorescence. All of the mutant cell lines selected for this study (shown in Table I) stained equally as well as the wild-type transfectant with either mAb 39B or 39J (9). Cells were maintained in culture in RPMI 1640 medium (Gibco Laboratories, Grand Island, NY) supplemented with 10% heat-inactivated FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, and 5×10^{-5} M 2-ME (RPMI/10).

T Cell Proliferation Assays. Transfected wild-type, mutant, and I-A^- M12.C3 cells (5×10^6 to 10^7 cells/ml) were treated with mitomycin C (Sigma Chemical Co., St. Louis, MO) at 40 μg/ml in RPMI/10 for 40 min at 37°C. Cells were washed four times and plated at 2×10^4 cells per well in 96-well, flat-bottomed, microtiter plates. The synthetic peptide rMBP 1-16, consisting of the acetylated NH_2-terminal 16 amino acids of rMBP, was prepared as previously described (11) and added to give a final concentration of between 0 and 133 μM. T cell clones were added at 2×10^4 cells per well in a final volume of 0.2 ml of RPMI/10. After 48 h, [^{3}H]thymidine was added at 1 μCi per well and cells were harvested 14–24 h later. The mean cpm of [^{3}H]thymidine incorporation was calculated for triplicate measurements and the standard deviations were <20% of the mean value. The experiments were repeated at least two times for each T cell clone and APC cell line. Each result is displayed as the stimulation index which is the cpm in the presence of antigen divided by cpm in the absence of antigen.

Results and Discussion

From the hypothetical model of class II MHC molecules (3), the mutations used for this study (Table I) would be predicted to fall on β-strands forming part of the floor or on an α-helix forming one wall of the proposed antigen binding cleft. Fig. 1 shows the results of an experiment in which three T cell clones were tested against the mutant cell lines over a wide range of antigen concentration. Clone 4R3.4 responded to peptide presented by mutants T.C, T.D, and T.9, as well as the wild-type T.A^k cell line. The mutant cell lines T.B, T.12, T.14, and T.17 presented antigen weakly, if at all, to clone 4R3.4. In contrast, mutant T.C did not present antigen to either clone 4R3.9 or AJ1.2. However, clone 4R3.9 responded well to peptide presented by T.9, moderately to T.D and significantly to T.12, T.14, and T.17. Clone AJ1.2 responded well to antigen presented by T.B, moderately to T.D, but not significantly to any of the other mutant cell lines.

Amino acid substitutions at positions 12 and 14 of the β chain caused a significant reduction in the antigen presenting ability of the I-A molecule for all of the clones tested. Previous studies had shown that the surface expression of mutated I-A on T.12 was slightly increased and T.14 decreased (15–20%) when compared with the wild-type transfectant (9). Cell line T.12 presented antigen to clone 4R3.9 alone (Fig. 1) and only at a 28-fold higher concentration of peptide (44.3 μM) than with the wild-type T-A^k transfectant. These results suggest that residues 12 and 14 of the β chain may quantitatively affect peptide binding. A recent study has indicated that residues 12 and 14 may also affect the binding of determinants recognized by al-

TABLE I

Cell Lines Expressing β Chains with Single Amino Acid or Block Substitutions and the Relationship of the Amino Acid Substitutions to rMBP 1-11 Responder (H-2k and H-2u) and Nonresponder Alleles (H-2d and H-2s)

Cell line	Residue	H-2k	H-2d	H-2u	H-2s	Predicted‡ position
T.9	9	H* →	V	V	F	Strand 1
T.12	12	Q →	K	Q	K	Strand 1
T.14	14	F →	E	F	E	Strand 1
T.17	17	F →	Y	F	F	Strand 1-2
T.B	63	K →	S	K	K	Helix 1-2
	65	Y →	PEI	Y	Y	
T.C	75	L →	V	L	V	Helix 2-3
	78	V →	A	V	V	
T.D	85	K	G	E	G	
	86	T →	P	T	V	Helix 3
	89	P	S	P	H	

* Amino acids are designated in the single-letter code.

‡ The predicted positions in the three-dimensional structure of I-A are from reference 3.

loreactive T cells (12). Two of three I-A^k-specific hybridomas responded to cell lines expressing an I-A^k β chain with combined mutations in regions B, C, and D. However, neither hybridoma responded to cell lines T.12 or T.14.

Table II summarizes the proliferative responses of five T cell clones. Data for each clone represent the peak response for each mutant APC in a single experiment and are representative of the response patterns from at least two separate experiments. All of the cell lines expressing mutated I-A molecules were able to present rMBP 1-16 to at least one of the T cell clones. T clones 4R3.4, 4R3.7, and 4R3.9 were similar in their elevated response to peptide presented by cell line T.9. However, each clone displayed a unique response pattern. 4R3.4 responded to both mutants T.9 and T.C, 4R3.7 responded to T.9 and T.B, while 4R3.9 did not respond significantly to peptide presented by either T.B or T.C. Clones AJ1.2 and 4R3.6 have consistently displayed an elevated response to peptide presented by cell lines T.B and T.17, respectively.

Substitution of histidine with valine at position 9 of the β chain resulted in improved presentation to three of the five clones tested (Table II). For clones 4R3.4 and 4R3.9 there was an increase in stimulation index compared with the wild-type T.A^k molecule, but there was not a significant shift in the dose-response curve (Fig. 1). This and the fact that clones AJ1.2 and 4R3.6 did not show an increased response to peptide presented by T.9 would argue against a straightforward effect of this substitution on peptide binding affinity. Even so, it is possible that changing histidine, a basic residue, to valine, an uncharged hydrophobic residue, favors the binding of the basic rMBP 1-16 peptide.

How can the heterogeneity in response patterns of the different T cell clones be explained? First, the results could indicate direct interaction of particular I-A residues

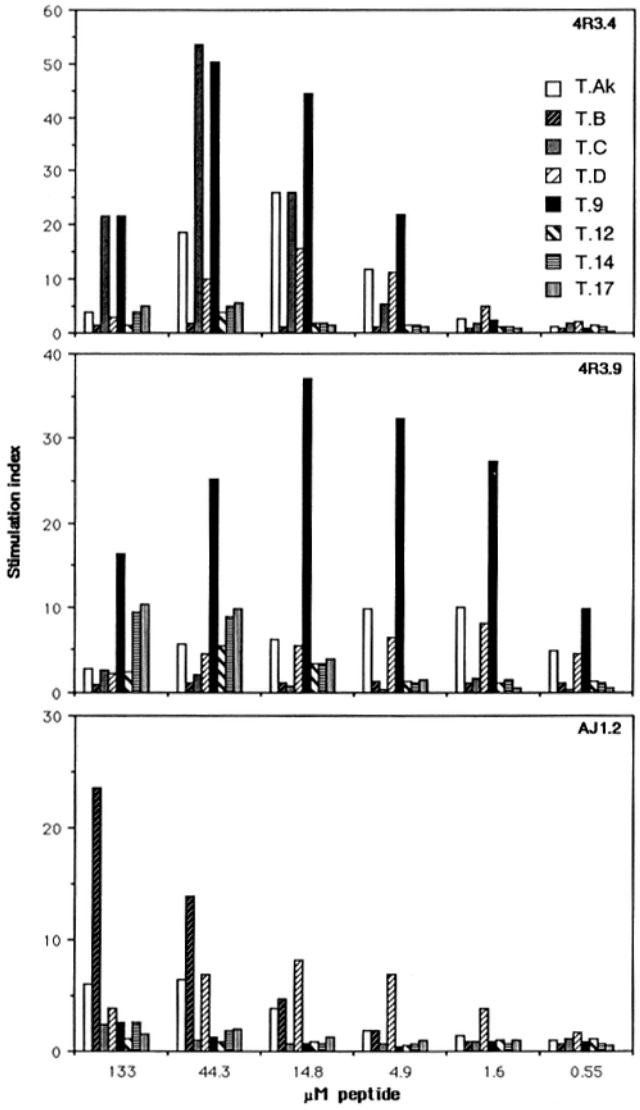

FIGURE 1. The proliferative response of rMBP peptide 1-9-specific and I-A^k-restricted T cell clones to rMBP peptide 1-16 presented by wild-type ($T.A^k$) and MHC-mutant cell lines. Data are expressed as "stimulation index" (mean cpm incorporation in the presence divided by mean cpm incorporation in the absence of antigen) in order to normalize for variation in background thymidine incorporation by the APCs alone. Mean cpm incorporation for 2×10^4 APC + 2×10^4 cloned T cells, in the absence of antigen, was calculated and is shown with the standard deviation in parentheses: $T.A^k$ 5,981 (1,264); T.B, 3,338 (425); T.C, 1,973 (364); T.D = 9,644 (1,537); T.9 3,529 (705); T.12, 10,234 (348); T.14, 3,118 (39.9); T.17, 1,360 (184). To control for an effect of residual feeder cells in the T cell cultures, each T cell clone was tested for background proliferation with 133 μM rMBP 1-16 in the absence of APC. The ratio of response with/without antigen for 4R3.4 was 0.85, for clone 4R3.9 was 0.76, and for AJ1.2 was 1.82.

with the TCR of isolated T cell clones. For example, Lys-63 and Tyr-65 could be TCR interaction residues for 4R3.4, but not for AJ1.2. The mutation in cell line T.B substitutes these residues and would, according to this theory, affect recognition by 4R3.4 and not AJ1.2. Secondly, the amino acid substitutions in the mutated β chains of cell lines T.9, T.B, T.C, and T.D could alter the conformation of other distant TCR interaction residues. Finally, since there may be constraints on the conformation of peptides when bound to MHC molecules, it is conceivable that amino acid substitutions in polymorphic residues of the I-A^k β chain could affect the conformation of bound peptide. In this way, amino acid substitutions in the I-A molecule could alter the alignment of TCR interaction residues in the peptide itself.

Our results are consistent with previous studies of class II-restricted responses (4-8). In studying the presentation of peptides from hen egg white lysozyme or oval-

TABLE II

Summary of the Response of RMBP Peptide 1-11-Specific T Cell Clones to Peptide Presented by Wild-Type (T.A^k) and I-A^k β-Chain Mutant Cell Lines

	APC							
T clone	T.A^k	T.B	T.C	T.D	T.9	T.12	T.14	T.17
4R3.4	+ + +	−	+ + +	+ +	+ + +	±	+	+
4R3.7	+	+ +	−	−	+ + +	−	−	−
4R3.9	+	−	±	+	+ + +	+	+	+
AJ1.2	+	+ + +	±	+	±	−	±	−
4R3.6	+	±	−	±	−	−	+	+ +

(−) 0–2 × stimulation index, (±) 2–5 × stimulation index, (+) 5–10 × stimulation index, (+ +) 10–20 × stimulation index, and (+ + +) >20 × stimulation index. These data represent maximal stimulation indices for the range of antigen concentrations tested.

bumin, two groups have shown how mutations in the presenting I-A molecule can affect the response of individual T cell hybridomas without abolishing antigen binding (6, 7). Furthermore, implications from two other studies (4, 8) are that residue 29 of I-Eβ can be involved in antigen binding, while residues 67, 70, and 71 of I-Aβ are part of a critical region for interaction with the TCR. Assuming conformational similarity between I-E and I-A molecules, and combining these data with our own results, we anticipate that amino acid alterations from polymorphic regions 1 and 2, predicted to reside on strands 1 and 2 of the class II MHC β chain, may either affect antigen binding (8) or alter TCR interactions (Table II, mutant T.9). Changes in residues from polymorphic regions 3 and 4, predicted to lie on the α-helical, "exposed" region of the molecule would be more likely to affect TCR interaction without necessarily abolishing antigen binding (Table II, mutants T.B, T.C, and T.D).

Finally, it should be stressed that these studies have largely concentrated on amino acid substitutions in isolated regions of an MHC molecule. There is no doubt that a combination of such substitutions can have a major effect on peptide binding and thus on Ir gene phenomena (13). However, our results stress the importance of polymorphic residues in TCR interactions with an MHC-antigen complex at the level of antigen presentation. By expression of mutant I-A molecules in transgenic mice it will be possible to analyze the role of such polymorphic amino acids in thymic selection of T cells specific for self antigens, such as MBP. Such a study will have direct bearing on those autoimmune diseases where a close association with class II MHC alleles has been noted (14).

Summary

Proteins encoded by genes in the MHC are highly polymorphic. For class II proteins the highest level of polymorphism is found in distinct regions of variability, notably in the membrane-distal domains. To investigate the role of such residues in antigen presentation, we have tested cells transfected with wild-type or mutant I-A^k β chains for their ability to present the NH_2-terminal peptide of myelin basic protein to a panel of T cell clones. We were unable to detect a gross effect on peptide binding, in that all of the mutant cell lines presented antigen to at least one of the cloned T cells. However, the results imply that the more NH_2-terminal residues, particularly 12 and 14, are involved in peptide interactions. Mutations at these residues

presented antigen only at high antigen concentrations. Furthermore, residues of the more COOH-terminal regions appear to determine TCR interactions. Mutations in the predicted α-helical regions of the β chain affected antigen presentation without abolishing peptide binding.

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